



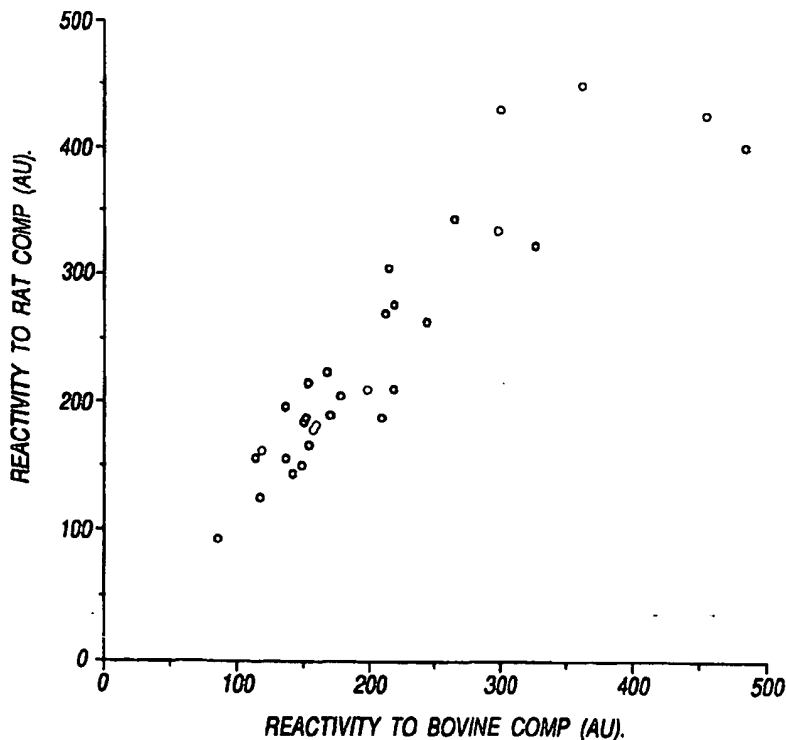
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(21) International Application Number: PCT/SE98/00682 (22) International Filing Date: 14 April 1998 (14.04.98) (30) Priority Data: 9701409-6 15 April 1997 (15.04.97) SE (71) Applicant (for all designated States except US): ASTRA AKTIEBOLAG (publ) [SE/SE]; S-151 85 Södertälje (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): HEINEGÅRD, Dick [SE/SE]; Lunds Universitet, Dept. of Cell and Molecular Biology, Sect for Connective-Tissue Biology, P.O. Box 94, S-221 00 Lund (SE). LORENTZEN, Johnny, C. [SE/SE]; Marielundsgatan 3A, S-753 25 Uppsala (SE). KLARESKOG, Lars [SE/SE]; Floragatan 8, S-752 18 Uppsala (SE). (74) Agent: ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.	

(54) Title: USE OF CARTILAGE OLIGOMERIC MATRIX PROTEIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

(57) Abstract

Use of cartilage oligomeric matrix protein (COMP) or fragments or analogs thereof for the manufacture of a pharmaceutical composition for prevention or treatment of arthritic conditions is described, wherein the pharmaceutical composition is administered in an amount effective to prevent or treat the arthritic condition.



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USE OF CARTILAGE OLIGOMERIC MATRIX PROTEIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

Background

The present invention relates to use of cartilage oligomeric matrix protein or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for the prevention or treatment of arthritic conditions in mammals.

Arthritis is a common name for several inflammatory joint diseases, e.g. rheumatoid arthritis, bacterial arthritis, reactive arthritis, and crystal-induced arthritis. Rheumatoid arthritis (RA) comprises a large group of non-bacterial conditions. Classical rheumatoid arthritis normally affects several joints (polyarthriti-
tis), but may also be restricted to a single joint (mono-
arthritis). The attack of the joint cartilage is only one of several factors which degenerate the cartilage and the bone and destroy the joint function. Also the joint capsule, the ligaments, and the bone tissue are affected.

Description of the Prior Art

At present, the symptoms of e.g. rheumatoid arthritis for some patients can be alleviated and the joint function be maintained or improved by medical treatment, e.g. with corticosteroids or methotrexat, physiotherapy, or surgical treatment. However, several patients are difficult or impossible to improve by treatment. Further, there exists no method for prevention of rheumatoid arthritis.

Thus, there is a need to prevent the symptoms of rheumatoid arthritis. There is also a need of further alleviating and reducing the symptoms of rheumatoid arthritis.

Summary of the Invention

An object of the present invention is to prevent or alleviate the symptoms of arthritic conditions. This object is achieved by use of cartilage oligomer matrix protein (COMP), or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for the

prevention or treatment of arthritic conditions in mammals. In a preferred embodiment of the present invention, the symptoms of rheumatoid arthritis are prevented or alleviated.

5 The present invention is based on the surprising observation made by the present inventors that COMP is arthritogenic. Rheumatoid arthritis is an autoimmune disease, i.e. it is considered to be due to a malfunction of the immune system of mammals wherein the immune system
10 fails to distinguish between foreign substances exposed to the mammal and the various endogenous substances of the mammal and begins to make antibodies to the latter.

 Cartilage oligomer matrix protein (COMP) is an 524 kDa oligomeric glycoprotein and is primarily present
15 in the upper parts of articular cartilage. In all types of cartilages it is synthesized by chondrocytes. The glycoprotein is composed of five identical sulphide-linked subunits with an apparent Mr of about 100 kDa each. It is markedly anionic, probably due to a high content
20 (about 30%) of aspartic and glutamic acid, and has also high contents of cysteine, giving an extensive disulphide bonding.

 COMP and its characteristics is extensively described in (1). Further, the nucleotide and the deduced amino
25 acid sequence of COMP is presented in (2). Furthermore, the model of COMP is shown in (8).

 As hydroxyproline has not been found in COMP, COMP does not contain any collagenous region. From the carbohydrate composition, the presence of N-linked, but not of
30 O-linked oligosaccharides, can be predicted. The presence of xylose, together with galactose amine and glucuronic acid in close to equimolar amounts, indicates substitution with chondroitin sulfate. The values of xylose, glucuronic acid and galactose amine suggests that each
35 COMP subunit carries 3-4 chondroitin sulfate chains of 14-15 disaccharides each.

Thus, COMP is a known compound and has previously been described as being useful in diagnostic methods for estimating the degree of cartilage degradation in arthritic patients (9).

5 It is already known that autoimmune reactions to certain cartilage antigens present in the joint cartilage can be used to induce rheumatoid arthritis in animal models. Collagen of different types (II, IX, and XI) and aggrecan have been classified as cartilage autoantigens
10 inducing autoimmunity and rheumatoid arthritis in animal models. These antigens have also been used in efforts to treat rheumatoid arthritis.

However, it has not previously been shown that COMP is arthritogenic and therefore potentially of use in the
15 treatment of rheumatoid arthritis. The inventors of the present invention have reached these results after experiments in which COMP has been added to rats. These experiments are described below.

The present invention therefore relates to the use
20 of cartilage oligomeric matrix protein (COMP), or fragments or analogues thereof, for the manufacture of a pharmaceutical composition for prevention or treatment of arthritic conditions in a mammal, wherein the pharmaceutical composition is administered in an amount
25 effective to prevent or treat the arthritic condition.

In one embodiment, the pharmaceutical composition comprises an RNA or DNA sequence capable of expressing the COMP or fragments or analogues thereof, or a vector cell containing said RNA or DNA sequences.

30 The pharmaceutical compositions of the present invention optionally also comprise one or more substances selected from the group consisting of collagene II, collagene IX, collagene XI, and aggrecan, or fragments or analogues thereof.

35 The COMP of use in the present invention may be of human, bovine, or rat origin, produced by microorganisms or chemically synthesized.

Figure relating to the Experiments

Fig. 1 shows the humoral reactivity to bovine COMP and rat COMP in sera from rats immunized with bovine COMP. An ELISA procedure was used to assess the humoral reactivity to COMP in sera from LEW.1AV1 (o) and F344 (•) rats, 16 and 38 days after immunization with 50-150 µg/rat of bovine COMP.

Experiments

The rat strains LEW.1AV1(DA) and F344 were originally derived from Zentralinstitut für Versuchstierzucht, Hannover, Germany. Characteristics and genetics of these strains are described in Genetic monitoring of inbred strains of rats (3). All strains were bred at the Biomedical Center in Uppsala and housed there or at the Karolinska Hospital under specific pathogen-free conditions. They were free from pathogens as determined by a health monitoring program for rats (Nov. 1995) at the National Veterinary Institute in Uppsala, Sweden. The rats were kept in a 12 h light/dark cycle, housed in polystyrene cages containing wood shavings and had free access to water and rodent chow. Rats used in experiments were females aged 10-19 weeks. All procedures involving animals were performed according to the guidelines provided by the central board for animal experiments at the Swedish Department of Agriculture. The experiments were approved by the Ethical board for animal experiments in Stockholm-North.

Induction of arthritis with bovine COMP and rat CII

Induction of arthritis with COMP was performed as follows: bovine COMP, prepared from cartilage (1), was dissolved in phosphate buffered saline (PBS) pH 7.4 at a concentration of 0.5 or 1.5 mg/ml. This solution was emulsified with an equal volume of incomplete Freund's adjuvant (Difco, Detroit) and 200 µl of the emulsion was injected intradermally at the base of the tail (50 or 150 µg/rat respectively). Induction of arthritis with collagen was performed as follows: rat collagen type II

(CII), prepared from a chondrosarcoma (4, 5), was dissolved in 0,1 M acetic acid at a concentration of 1.5 mg/ml. This solution was emulsified with an equal volume of incomplete Freund's adjuvant and 200 µl of the emulsion was injected intradermally at the base of the tail (150 µg/rat).

Evaluation of arthritis

Arthritis was assessed using a scale from 0-16, each of four paws being scored from 0-4 in which 0=no arthritis, 1-3=a summary of points as follows: swelling of the ankle-1 point, swelling of one or more intratarsal and/or metatarsal joints-1 point, and swelling of one or more interphalangeal joints-1 point, 4=swelling of all joints, i.e. the entire paw. The rats were examined every second to fourth day from day 10 until day38 after immunization.

Preparation of draining lymph node cells

Inguinal lymph node cells from rats immunized with COMP and from non-immunized control rats were obtained by dissection of the lymph nodes and gentle grinding through a tea-strainer. The cell suspensions were washed three times in PBS pH 7.4.

Measurement of cellular reactivity to bovine COMP: Proliferation

The inguinal lymph node cells were suspended at 1×10^6 cells/ml in DMEM (X) supplemented with 5% FCS (Hyclone, Logan, UK), penicillin (100 u/ml), glutamine (2 mM) and streptomycin (100 µg/ml), all from Sigma, St Louis, MO. Cells from individual animals were plated in 96-well round-bottom cell culture plates (Nunc, Roskilde, Denmark), 0.2 ml per well. Antigens were added to triplicates of wells for each animal, dissolved in 10 µl PBS pH 7.4, to give the final concentrations: COMP, 10 µg/ml and 50 µg/ml, or ConA 5 µg/ml. As control, 10 µl of PBS were added to the wells. The cells were incubated for 96 hours in 37°C and 5% CO₂, and cell proliferation assessed by labelling proliferating cells in each well with 1 µCi of ³H-thymidine for the final 6 hours before cell

harvest. Incorporation of label was determined by liquid scintillation counting.

Purification of rat COMP

Rat COMP was prepared from Swarm rat chondrosarcoma
5 (6). Briefly, frozen Swarm rat chondrosarcoma was homogenized in PBS containing 1 mM phenylmethanesulfonyl fluoride (PMSF) and 2 mM N-ethylmaleimide (NEM) followed by centrifugation for 30 min at 15 000 g. The homogenization and centrifugation was repeated twice, followed by
10 extraction of COMP in the same buffer containing in addition 10 mM EDTA. The EDTA extract was diluted 1+1 in water and applied to an anion exchange column (20x1.6 cm) of DEAE Sepharose CL-6B (Pharmacia LKB Biotechnology) equilibrated with 10 mM tris containing 2 mM EDTA. The
15 column was eluted with a gradient of 0-1.0 M NaCl in the same buffer. COMP containing fractions were concentrated by ultrafiltration (YM-10 filter, Amicon) and applied to a gel filtration column (50 x 1.6 cm) of Superose 6 (Pharmacia LKB Biotechnology), and eluted with PBS
20 containing 2 mM EDTA.

Quantitation of humoral anti-bovine COMP, anti-rat COMP and anti-rat CII immunity

IgG antibody titers to COMP or CII were determined using an enzyme-linked immunosorbent assay (ELISA).
25 Maxisorp Micro-ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 10 µg/ml of rat CII or 2-10 µg/ml of COMP in PBS pH 7.4. Individual sera were diluted, added and bound antibody was detected by incubation with alkaline phosphatase-conjugated goat anti-rat
30 IgG, specific for heavy and light chains (Jackson Immuno-research Laboratories, West Groove, PA). The subsequent quantitation of bound enzyme was performed with a p-nitrophenyl-containing substrate buffer in an E-max spectrophotometer (Molecular Devices, Sunnyvale, CA). Data are
35 presented as antibody units (AU) per ml, calculated by comparison with the linear portion of a standard curve using positive and pooled anti-CII serum or anti-COMP

serum from LEW.1AV1 rats with given arbitrary values of 100 AU/ml respectively. Each plate contained positive reference serum, serum samples to be measured and negative serum controls in duplicates. Negative serum controls were individual sera from non-immunized LEW.1AV1 rats.

RESULTS

Arthritogenicity of bovine COMP in LEW.1AV1 and F344 rats

In the first experiment, six LEW.1AV1 rats were immunized with 50 µg COMP/rat (table 1). 15-19 days later, three of these (3/6) developed arthritis which mainly affected the hind ankles. The disease was self-limiting, there were no macroscopic signs of arthritis or ankylosis 38 days after immunization. In the second experiment, nine LEW.1AV1 rats and four age-matched F344 rats were immunized with 150 µg COMP/rat (Table 1). This higher dose of COMP resulted in a similar incidence of arthritis in the LEW.1AV1 rats (5/11) but the disease appeared earlier, 12-14 days after immunization. Furthermore, the arthritis was more severe and typically affected ankle joints and metatarsal joints in the hind paws, arthritis in finger joints and fore paws was also observed. Two rats were monitored clinically until 38 days after immunization at which time they had signs of ankylosis. In contrast to rats of the LEW.1AV1 strain, F344 rats appeared less susceptible to COMP-induced arthritis since only minimal signs of disease could be detected, at a late time-point 25-29 days after immunization, in one out of four rats.

Humoral reactivity to bovine COMP in individual LEW.1AV1 and F344 rats, 16 days after immunization with bovine COMP

Analysis of individual sera demonstrated humoral reactivity to COMP in both F344 rats and LEW.1AV1 rats (Table 1). There was no correlation between presence of antibodies and arthritis.

Humoral cross-reactivity between bovine COMP and rat COMP

Sera from LEW.1AV1 and F344 rats, immunized with bovine COMP, were tested for humoral reactivity to bovine COMP and rat COMP (Figure 1). The reactivity to bovine
5 COMP correlated well to that against rat COMP in both rat strains, which demonstrates that the humoral immune response induced by immunization with bovine COMP is auto-immune.

Humoral reactivity to COMP and CII in LEW.1AV1 rats with
10 arthritis induced by immunization with COMP or CII

Sera from LEW.1AV1 rats immunized with incomplete Freund's adjuvant alone, or together with bovine COMP or rat CII respectively, were tested for humoral reactivity to both bovine COMP and rat CII. The results depicted in
15 Table 2 demonstrate that reactivity to COMP and CII develops in LEW.1AV1 immunized with the respective antigens, and that there is no cross-reactivity between these two antigens. Interestingly, there were no signs of an anti-CII reactivity in rats with COMP-induced arthritis.
20 No or minimal reactivity to COMP was detected in rats with CII-induced arthritis.

Two features of the humoral and cellular immune response in arthritic animals were of principal interest. Firstly, the humoral reactivity to COMP was apparently
25 not accompanied by reactivity to rat collagen type II (CII). Thus, there is no evidence for immunological cross-reactivity between these two arthritogenic antigens, neither is there evidence for spreading of the humoral autoimmune response to rat CII. Secondly, a
30 marked proliferative cellular immune response towards COMP was evident. This implies that the nature of T-cell mediated immune reactions towards COMP is more amenable to study than those to previously described cartilage autoantigens, such as rat CII which induces little or no
35 in vitro T-cell proliferation (unpublished results).

In this study it was also demonstrated that while the LEW.1AV1 strain were permissive for COMP-induced

arthritis, F344 rats appeared resistant. This indicates that susceptibility is genetically determined.

COMP is of interest as an arthritis-inducing antigen for several reasons. Firstly, COMP is present in the most superficial parts of the articular cartilage where it may be accessible to immune cells in the joints. Secondly, COMP is released into synovial fluid and serum of patients with early destructive arthritis (7), implying that this protein is also accessible to circulating immune cells. It has not earlier been reported whether an induced autoimmunity against COMP can cause arthritis such as in this present study, nor whether autoimmune reactions towards COMP occur in patients with arthritis.

Bovine and rat COMP used in these experiments were purified from cartilage such that no contamination of other proteins could be detected, as observed by the absence of other proteins than COMP on SDS-PAGE gels used for the biochemical characterization (data not shown). However, the existence of minor contaminants in the preparation may not be excluded, but such contaminants are unlikely to be responsible for arthritis induction since their concentrations would be orders of magnitude lower than that of other arthritogens used in rats. That contaminating collagen type II is not involved in arthritis development is also indicated by the lack of a humoral immune response to rat CII in rats immunized with the COMP preparation.

Table. 1. Development of arthritis and humoral reactivity to COMP in LEW.1AV1 rats and F344 rats after immunization with COMP.

Rat Strain	#	Dose of COMP (µg/rat)	Day of Onset	Maximal Severity 0-38 dpi†	Severity 38 dpi	α-COMP (AU) 18 dpi	α-COMP (AU) 38 dpi.
LEW.1AV1 (6)	a1	50	15	1	0	nd**	219
	a2	50	-	0	0	nd	153
	a3	50	-	0	0	nd	213
	a4	50	-	0	0	nd	114
	a5	50	19	2	0	nd	215
	a6	50	19	3	0	nd	326
LEW.1AV1 (11)	b1*	150	12-14	2	na††	155	na
	b2*	150	12-14	5	na	152	na
	b3	150	-	0	0	210	219
	b4*	150	12-14	2	na	117	na
	b5	150	-	0	0	148	168
	b6	150	12-14	4	2	137	265
	b7	150	12-14	7	6	142	483
	b8	150	-	0	0	151	243
	b9	150	-	0	0	85	178
	b10*	150	-	0	na	136	na
	b11*	150	-	0	na	170	na
F344 (4)	b12	150	-	0	0	157	298
	b13	150	-	0	0	199	300
	b14	150	-	0	0	160	381
	b15	150	26-29	1	0	118	453

LEW.1AV1 and F344 rats were immunized with bovine COMP in incomplete Freund's adjuvant, the humoral reactivity was tested against bovine COMP by an ELISA procedure. #=animal number. †dpi=days post-immunization. *these rats were killed 16 dpi. **nd=not done. ††na=not applicable because rats were killed 16 dpi.

Table 2. Humoral reactivity to COMP and collagen type II (CII) in LEW.1AV1 rats immunized with incomplete Freund's adjuvant (IFA) alone, or together with COMP or CII.

Rats immunized with:	Maximal severity	α -CII (AU)	α -COMP (AU)
IFA (6)	0	0	0
CII+IFA (5)	7-12	61-223	0-2
COMP+IFA (5)	1-7	0	215-483

Sera were obtained from rats which developed bovine COMP-induced arthritis (38 days post immunization, dpi), rat collagen-induced arthritis (28 dpi) or, as a control, from rats immunized with IFA (28 dpi).

Table 3. Proliferation of lymph node cells from LEW.1AV1 rats immunized with COMP, a comparison between unstimulated cells and cells stimulated with COMP or Con A.

Animal number	Arthritis Severity	Proliferation (cpm) stimulated by:		
		Control	COMP	Con A
1	2	1506	12433	26315
2	5	2754	27014	37624
3	2	4541	25604	38135
4	0	3658	28820	40961

Lymph node cells were prepared from four female LEW.1AV1 rats 16 days after immunization with 150µg/rat of bovine COMP emulsified in incomplete Freund's adjuvant. The cells were either unstimulated (control) or stimulated with bovine COMP (10 µg/ml) or with Con A (5 µg/ml).

The expression "fragments" of COMP used throughout the application include any partial amino acid sequences or modified partial sequences thereof, i.e. those being shorter in length than COMP and/or containing any deletions/substitutions or modifications to the constituent amino acids, that are capable of inducing a biological response similar to COMP, i.e. the ability to suppress or eliminate T-cell or B-cell mediated and/or T-cell or B-cell dependent autoimmune response, or act in a manner so as to inhibit the arthritogenic effects of COMP. The expression "analogues" of COMP used throughout the application includes compounds (both peptides and non-peptide chemical compounds) that possess similar biological activity to COMP, i.e. the ability to eliminate or suppress the T-cell or B-cell mediated and/or T-cell or B-cell dependent autoimmune response, or act in a manner so as to inhibit the arthritogenic effects of COMP. Thus, the latter expression includes amino acid sequences which differ from the amino acid sequence of COMP by one or more amino acids, while retaining substantially equivalent biological activity, as well as chemical compounds which mimic the biological activity of COMP in its ability to suppress or alleviate the symptoms of rheumatoid arthritis.

The pharmaceutical composition comprising COMP or the fragments or analogues thereof may be administered to a mammal, preferably a human, in a number of known routes including oral, mucosal, nasal, rectal or vaginal route, or by inhalation or injection.

For oral administration the pharmaceutical composition may be e.g. tablets, pills, capsules, syrups, powders, granules, or solutions.

For oral administration the active compound may be admixed with an adjuvant or a carrier, e.g. lactose, saccharose, sorbitol, mannitol, starches such as potato starch, corn starch or amylopectin, cellulose derivatives, a binder such as gelatine or polyvinylpyrrolidone,

and a lubricant such as magnesium stearate, calcium stearate, polyethylene glycol, waxes, paraffin, and the like, and then compressed into tablets. If coated tablets are required, the cores, prepared as described above, may
5 be coated with a concentrated sugar solution which may contain e.g. gum arabic, gelatine, talcum, titanium dioxide, and the like. Alternatively, the tablet may be coated with a suitable polymer dissolved in a readily volatile organic solvent.

10 For the preparation of soft gelatine capsules, the compound may be admixed with e.g. a vegetable oil or polyethylene glycol. Hard gelatine capsules may contain granules of the compound using either the above mentioned excipients for tablets, e.g. lactose, saccharose, sorbi-
15 tol, mannitol, starches, cellulose derivatives or gelatine. Also liquid or semisolid formulations of the drug may be filled into hard gelatine capsules.

Liquid preparations for oral application may be in the form of syrups or suspensions, for example solutions
20 containing the compound, the balance being sugar and a mixture of ethanol, water, glycerol and propylene glycol. Optionally such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethyl-cellulose as a thickening agent or other excipients known
25 to those skilled in art.

The pharmaceutical composition may also include pharmaceutically acceptable carriers, such as water, suspending agents, and emulsifying agents.

Also, mucosa binding compounds may be included in
30 the pharmaceutical composition.

The exact dosages of the active ingredient, i.e. COMP or the biological active fragments or analogues thereof, depend on the age, sex, and physical condition, i.e. the severity of rheumatoid arthritis, of the patient
35 and on any concurrent treatments. However, the dosage has to be such that the symptoms of rheumatoid arthritis are prevented or alleviated.

In another embodiment the pharmaceutical composition according to the present invention comprises COMP or fragments or analogues thereof in combination with one or more arthritogenic substances chosen from the group
5 consisting of collagene II, IX, XI, and aggrecan, or fragments or analogues thereof.

COMP, and fragments or analogs thereof, may be isolated from the naturally occurring material as described in (1) or may be expressed from the nucleotide
10 sequence given for COMP in (2). The skilled man will be aware as to how such expression may be obtained and how such fragments or analogs may be prepared from the nucleotide sequence. COMP and its fragments and analogs may also be chemically synthesized using the deduced
15 amino acid sequence given in (2).

The present invention therefore also relates to the use of COMP as described above, wherein the pharmaceutical composition comprises RNA or DNA sequences expressing COMP or fragments or analogues thereof, or a
20 vector cell containing said RNA or DNA sequences. Thus, the RNA or DNA sequences are administered to the mammal either directly or via the vector cell.

COMP or the fragments or analogues thereof may have any origin, but has preferably human, rat, or bovine
25 origin. It may be prepared by purification from any of these origins by means of standard methods. It can also be expressed by a microorganism or be chemically synthesized, as described above.

According to the present invention COMP or fragments
30 or analogues thereof may be added in a pharmaceutical composition administration to mammals in order to prevent or alleviate the symptoms of arthritic conditions, preferably rheumatoid arthritis. Thus, when the pharmaceutical composition according to the present
35 invention is administered to a mammal, preferably a human, having rheumatoid arthritis, COMP or the fragments

or analogues thereof acts as a kind of tolerogen and thereby induces immunological tolerance.

Further, COMP or fragments or analogues thereof may also be used to prevent arthritic conditions, preferably
5 rheumatoid arthritis by administration to a mammal suspected to develop such arthritic conditions, e.g. due to genetical predictions. In such a case, the disturbed balance due to autoimmunological reactions will never appear, nor the arthritic condition.

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CLAIMS

1. Use of cartilage oligomeric matrix protein
5 (COMP), or fragments or analogues thereof, for the
manufacture of a pharmaceutical composition for the
prevention or treatment of arthritic conditions in a
mammal, wherein the pharmaceutical composition is
10 administrated in an amount effective to prevent or treat
the arthritic condition.
- ~~2. Use according to claim 1, wherein the pharmaceu-~~
tical composition comprises an RNA or DNA sequence
capable of expressing COMP or fragments or analogues
thereof, or a vector cell containing said RNA or DNA
15 sequences.
3. Use according to claim 1 or 2, wherein in the
pharmaceutical composition optionally also comprises one
or more substances selected from the group consisting of
collagene II, collagene IX, collagene XI, and aggrecan,
20 or fragments or analogues thereof.
4. Use according to claim 1, wherein the COMP has
human, bovine, or rat origin, or has been produced by
microorganisms or has been chemically synthesized.
5. Use according to any one of the preceding claims,
25 wherein the pharmaceutical composition is administrated
by the oral, mucosal, nasal, rectal or vaginal route, or
by inhalation or injection.
6. Use according to claim 1, wherein the mammal is a
human.
- 30 7. Use according to claim 1, wherein the arthritic
condition is rheumatoid arthritis.

1/1

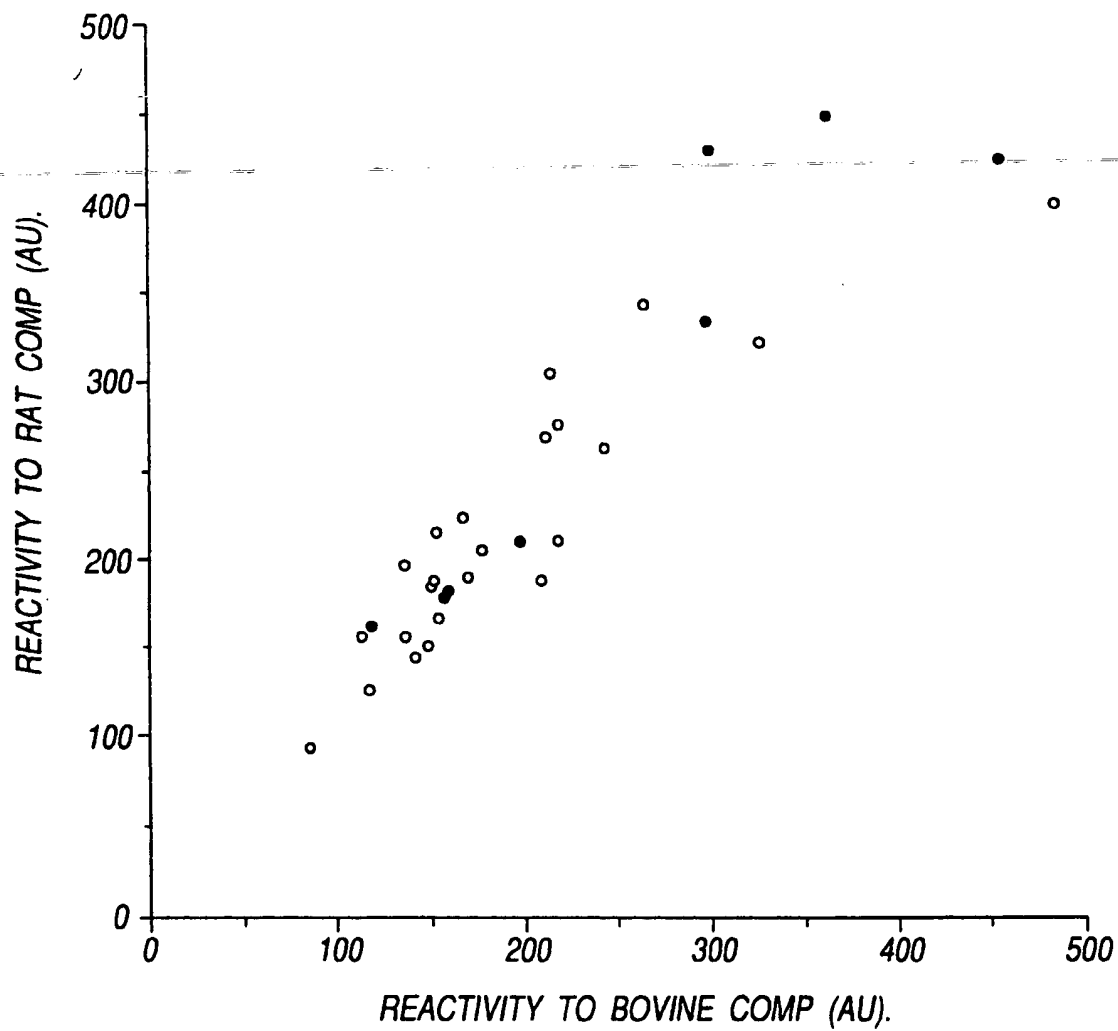


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00682

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/17, C07K 14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, US PATENTS FULLTEXT, REGISTRY, MEDLINE, BIOSIS, EMBASE, DIALINDEX,
(ALLSCIENCE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9415627 A1 (THE GENERAL HOSPITAL CORPORATION), 21 July 1994 (21.07.94), page 2, line 6 - line 16, the claims --	1-7
A	Dialog Information Services, file 155, Medline, Dialog Accession no. 08369593, Briggs MD et al: "Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene", Nat Genet (UNITED STATES) Jul 1995, 10 (3) p330-6 --	1-7
A	WO 9110424 A1 (NORTHWESTERN UNIVERSITY), 25 July 1991 (25.07.91), page 13, line 17 - line 18 --	1-7



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 June 1998

Date of mailing of the international search report

02-07-1998

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00682

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>Dialog Information Services, file 155, Medline, Dialog accession no. 07153808, Medline accession no. 92404864, Forslind K. et al: "Increased serum concentrations of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis", Br J Rheumatol (ENGLAND) Sep 1992, 31 (9) p593-8</p> <p style="text-align: center;">-- =====</p>	1-7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00682

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-7
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next page

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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The wording "analogues thereof" of claim 1 is not considered to be clear and concise (c.f. PCT Article 6).

The "analogues" are not considered to have been restricted to compounds having the same special technical feature as COMP, which feature is responsible for the effect on arthritic conditions. No examples of the intended COMP analogues have been given in the application.

Therefore, the search has been restricted to those analogues that are, in respect of its amino acid/nucleotide sequence very similar to COMP.

INTERNATIONAL SEARCH REPORT
Information on patent family members

09/06/98

International application No.

PCT/SE 98/00682

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9415627 A1	21/07/94	AU 6228494 A CA 2153241 A EP 0679089 A JP 8507205 T	15/08/94 21/07/94 02/11/95 06/08/96
WO 9110424 A1	25/07/91	AT 126065 T CA 2050290 A DE 69111919 D,T DK 463152 T EP 0463152 A,B SE 0463152 T3 JP 4504429 T US 5192744 A	15/08/95 13/07/91 07/12/95 06/11/95 02/01/92 06/08/92 09/03/93